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Published in:
Online Proceedings

Publication date:
2016

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Holkenbrink, C., Beder, J., Dam, M. I., & Borodina, I. (2016). A toolbox for accelerating strain engineering of oleaginous yeast *Yarrowia lipolytica*. In *Online Proceedings* American Institute of Chemical Engineers.

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A Toolbox for Accelerating Strain Engineering of Oleaginous Yeast *Yarrowia lipolytica*

Holkenbrink, C., Beder, J., Dam, M.I., and I. Borodina

The non-conventional oleaginous yeast *Y. lipolytica* is valued for its capability to accumulate high amounts of storage lipids and to degrade hydrophobic substrates. It is an attractive host for the production of lipids and fatty acid-derived compounds and for bioremediation, however the strain development is hampered by the lack of genetic tools. We seek to develop a genome editing toolbox, which will allow rapid iterative combinatorial strain engineering (Figure 1), i.e., repeatedly introducing combinations of multiple edits, such as gene knock-ins/knock-outs. First, we developed a set of standardized integration vectors for gene overexpression from high expression genome spots. Second, we adopted CRISPR-Cas9 technology for *Y. lipolytica* to enable genome editing with high efficiency without the need of a selection marker. We demonstrate the utility of the toolbox by engineering a strain for production of a potential platform chemical, α -ketoglutarate, from renewable feedstocks.